searching both groups would not be a serious burden for the examiner...a search of the p100 literature would locate p100 monoclonal antibodies and vis versa." The Examiner has not found the ground persuasive because the inventions are classified in entirely different areas of the patent literature. The Examiner stated that the requirement is still deemed to be proper and is therefore made FINAL.

The Examiner stated that the disclosure is objected to because of the following informalities: the listing of related applications in the specification is incomplete. The Examiner pointed out that only serial nos. 07/297,188, 07/182,501 and 06/871,102 are listed and there is at least one other application (07/412,668) which is not mentioned. The Examiner required appropriate correction.

In response to the Examiner's objection to the disclosure because of informalities, applicants have amended the specification to include a listing of the related applications.

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

On page 2 of the October 31, 1995 Office Action, the Examiner objected to the specification under 35 U.S.C. § 112, first paragraph, as failing to provide an adequate written description of the invention and failing to provide an enabling disclosure.

On page 3 of the October 31, 1995 Office Action, the Examiner stated that according to the specification, the existence of p100 was noted when antibody raised against cell lysates recognized p185 in cell lysates, and also recognized a smaller related protein in cell culture supernatants and biological fluids. The Examiner stated that there is very little discussion of the physical and biological properties of p185 or p100 beyond this, therefore, the

availability of at least one of the disclosed antibodies to the external domain of p185 must be assured to enable one of the skill in the art to isolate and /or identify p100 without undue experimentation.

The Examiner also pointed out that the specification lacks complete deposit information for the deposit of the hybridoma cell lines which secrete monoclonal antibodies OD3, NB-3 and TA-1. The Examiner stated that because it does not appear that any of these antibodies are known and publicly available or can be reproducibly isolated from nature without undue experimentation and because the only disclosed means of isolating and/or identifying p100 requires the use of an antibody specific for the external domain of p185, a suitable deposit of any one of the disclosed hybridoma cell lines is required for patent purposes.

On page 3 of the October 31, 1995 Office Action, the Examiner stated that applicants' referral to the deposit of OD3, NB-3 and TA-1 as HB 10204, HB 10205 and HB 10206 on page 9 of the specification is an insufficient assurance that all of the conditions of 37 C.F.R. § 1.801 through § 1.809 have been met. The Examiner further stated that because the deposits were made under the provisions of the Budapest Treaty, filing of an affidavit or declaration by applicants, assignees or a statement by an attorney of record over his or her signature and registration number stating that the deposits have been accepted by an International Depository Authority under the provisions of the Budapest Treaty, that all restrictions upon public access to the deposits will be irrevocably removed upon the grant of a patent on this application and that the deposit will be replaced if viable samples cannot be dispensed by the depository is required. The Examiner maintained that this requirement is necessary when deposits are made under the provisions of the Budapest Treaty as the Treaty leaves these

specific matters to the discretion of each State. The Examiner stated that an amendment of the specification to recite the date of the deposit and the complete name and address of the depository is required.

On page 4 of the October 31, 1995 Office Action, the Examiner stated that claims 1 and 2 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

In response to the Examiner's objection to the specification under 35 U.S.C. § 112, first paragraph, applicants point out the following. One, the monoclonal antibodies OD3, NB3 and TA1 specifically recognize the extracellular domain of neu, i.e. p100. Second, applicants draw the Examiner's attention to ATCC Receipt In The Case Of An Original Deposit Issued Pursuant to Rule 7.3 and Viability Statement Issued Pursuant to Rule 10.2, dated August 16, 1989 attached hereto as Exhibit A. The hybridoma cell lines which secrete monoclonal antibodies OD3, NB-3 and TA-1, were given ATCC designations HB 10204, HB 10205 and HB 10206, respectively. The deposits were received and accepted by ATCC on August 11, 1989 and were found to be viable. Applicants point out that the date of deposit and the name and address of the depository can be found on page 9, lines 2-18 of the specification.

The undersigned attorney of record herewith states that the deposits have been accepted by an International Depository Authority under the provisions of the Budapest Treaty, that all restrictions upon public access to the deposits will be irrevocably removed upon the grant of a patent on this application and that the deposit will be replaced if viable samples cannot be dispensed by the depository is required.

Thus, applicants point out that the hybridoma cell lines are available through the ATCC in response to the Examiner's rejection of claims 1 and 2 for the reasons set forth in the objection to the specification. Accordingly, applicants respectfully request the Examiner reconsider and withdraw the rejection under 35 U.S.C. §112, first paragraph.

REJECTION UNDER 35 U.S.C. §112, SECOND PARAGRAPH

On page 4 of the October 1995 Office Action the Examiner further stated that claims 1 and 2 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner contended that the recitation "corresponds substantially to" is vague and indefinite. The Examiner points out that the specification states that this term "provides for conservative additions, deletions and substitutions;" this is taken to mean conservative amino acid substitutions, but it still does not explain how much can be deleted from the protein without altering its identity, or how many amino acids can be added (p185 "corresponds substantially to" p100 by this definition).

In response to Examiner's comment regarding the terms "corresponds substantially to", applicants point out that conservative amino acid substitutions, additions and deletions encompasses the replacement, addition or deletion of one or more amino acids which do(es) not alter the biochemical nature of the peptide and allows the peptide to retain substantially the same structural and biochemical features. One skilled in the art would be able to predict which amino acids may be substituted, added or deleted based upon the active sites or binding sites of the peptide as well

American Type Culture Collection
12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)881-2600 Telex: 898-055 ATCCNORTH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Walter P. Carney
E. I. Dupont 500-2
331 Treble Cove Road
North Billerica, Massachusetts 01862

Deposited on Behalf of: Walter P. Carney

Identification Reference by Depositor:	ATCC Designation
Hybridoma, OD-3	HB 10204
Hybridoma, NB3	HB 10205
Hybridoma, TA-1	HB 10206

The deposits were accompanied by: ___ a scientific description ___ a proposed taxonomic description indicated above.

The deposits were received August 11, 1989 by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

X We will inform you of requests for the strains for 30 years.
___ We will not inform you of requests for the strains.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested August 15, 1989. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC: Bobbie A. Brandon
(Mrs.) Bobbie A. Brandon, Head, ATCC Patent Depository

Date: August 16, 1989

cc: Lynne Christenbury

Form BP 4/9

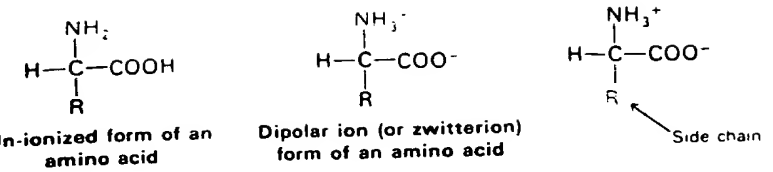


Figure 2-5
Structure of the un-ionized and zwitterion forms of an α-amino acid.

distinctive R group bonded to a carbon atom, which is called the α-carbon because it is adjacent to the carboxyl (acidic) group (Figure 2-5). An R group is referred to as a *side chain* for reasons that will be evident shortly.

Amino acids in solution at neutral pH are predominantly *dipolar ions* (or *zwitterions*) rather than un-ionized molecules. In the dipolar form of an amino acid, the amino group is protonated ($-NH_3^+$) and the carboxyl group is dissociated ($-COO^-$). The ionization state of an amino acid varies with pH (Figure 2-6). In acid solution (e.g., pH 1), the carboxyl group is un-ionized (—COOH) and the amino group is ionized ($-NH_3^+$). In alkaline solution (e.g., pH 11), the carboxyl group is ionized ($-COO^-$) and the amino group is un-ionized ($-NH_2$). For glycine, the pK of the carboxyl group is 2.3 and that of the amino group is 9.6. In other words, the midpoint of the first ionization is at pH 2.3, and that of the second is at pH 9.6. For a review of acid-base concepts and pH, see the Appendix to this chapter.

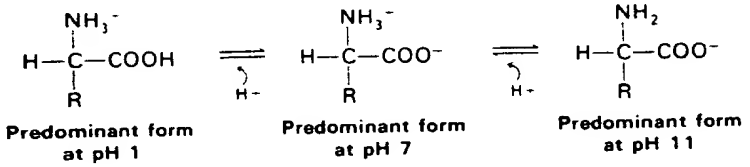


Figure 2-6
Ionization states of an amino acid depend on pH.

The tetrahedral array of four different groups about the α-carbon atom confers optical activity on amino acids. The two mirror-image forms are called the L-isomer and the D-isomer (Figure 2-7). *Only L-amino acids are constituents of proteins.* Hence, the designation of the optical isomer will be omitted and the L-isomer implied in discussions of proteins herein, unless otherwise noted.

Twenty kinds of side chains varying in *size, shape, charge, hydrogen-bonding capacity,* and *chemical reactivity* are commonly found in proteins. Indeed, all proteins in all species, from bacteria to humans, are constructed from the same set of twenty amino acids. This fundamental alphabet of proteins is at least two billion years old. The remarkable range of functions mediated by proteins results from the diversity and versatility of these twenty kinds of building blocks. We shall explore ways in which this alphabet is used to create the intricate three-dimensional structures that enable proteins to carry out so many biological processes.

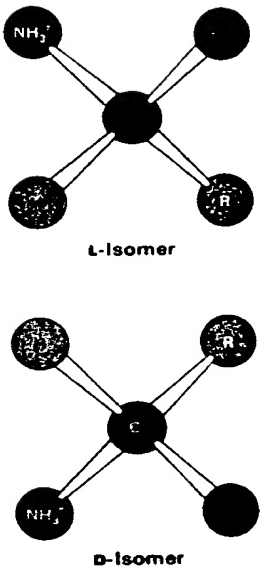


Figure 2-7
Absolute configurations of the L- and D-isomers of amino acids. R refers to the side chain.

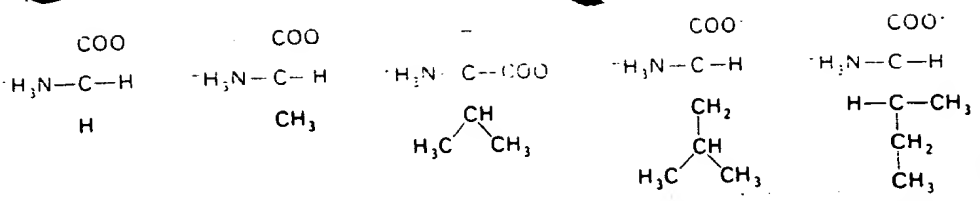


Figure 2-8
Amino acids having aliphatic side chains.

Let us look at this repertoire of amino acids. The simplest one is *glycine*, which has just a hydrogen atom as its side chain (Figure 2-8). *Alanine* comes next, with a methyl group as its side chain. Larger hydrocarbon side chains (three and four carbons long) are found in *valine*, *leucine*, and *isoleucine*. These larger aliphatic side chains are *hydrophobic*—that is, they have an aversion to water and like to cluster. As will be discussed later, the three-dimensional structure of water-soluble proteins is stabilized by the coming together of hydrophobic side chains to avoid contact with water. The different sizes and shapes of these hydrocarbon side chains (Figure 2-9) enable them to pack together to form compact structures with few holes.

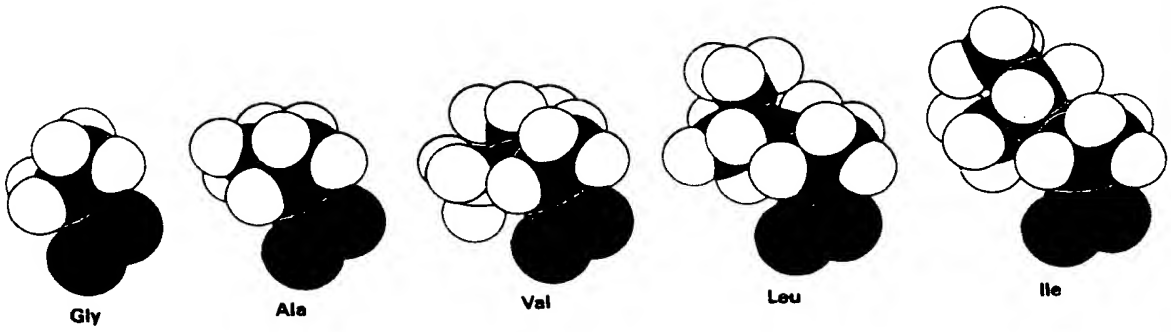


Figure 2-9
Models of aliphatic amino acids

Proline also has an aliphatic side chain but it differs from other members of the set of twenty in that its side chain is bonded to both the nitrogen and α-carbon atoms. The resulting cyclic structure (Figure 2-10) markedly influences protein architecture. Proline, often found in the bends of folded protein chains, is not averse to being exposed to water. Note that proline contains a secondary rather than a primary amino group, which makes it an *imino* acid.

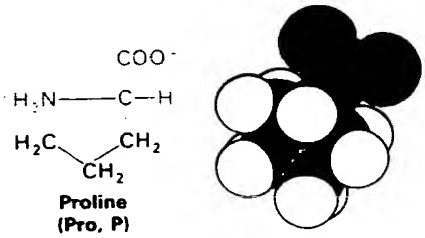


Figure 2-10
Proline differs from the other common amino acids in having a secondary amino group.

Three amino acids with *aromatic side chains* are part of the fundamental repertoire (Figure 2-11). *Phenylalanine*, as its name indicates, contains a phenyl ring attached to a methylene (—CH_2—) group. *Tryptophan* has an indole ring joined to a methylene group; this side chain contains a nitrogen atom in addition to carbon and hydrogen atoms.

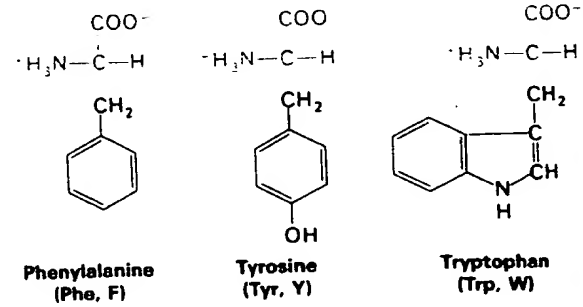


Figure 2-11
Phenylalanine, tyrosine, and tryptophan have aromatic side chains.

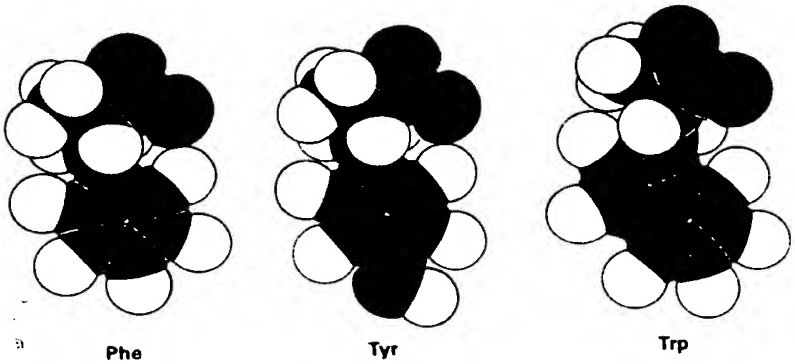


Figure 2-12
Models of the aromatic amino acids.

Phenylalanine and tryptophan are highly hydrophobic. The aromatic ring of *tyrosine* contains a hydroxyl group, which makes tyrosine less hydrophobic than phenylalanine. Moreover, this hydroxyl group is reactive, in contrast with the rather inert side chains of all the other amino acids discussed thus far. The aromatic rings of phenylalanine, tryptophan, and tyrosine contain delocalized pi-electron clouds that enable them to interact with other pi-systems and to transfer electrons.

A *sulfur atom* is present in the side chains of two amino acids (Figure 2-13). *Cysteine* contains a sulfhydryl group (—SH) and *methionine* contains a sulfur atom in a thioether linkage (—S—CH_3). Both of these sulfur-containing side chains are hydrophobic. The sulfhydryl group of cysteine is highly reactive. As will be discussed shortly, cysteine plays a special role in shaping some proteins by forming disulfide links.

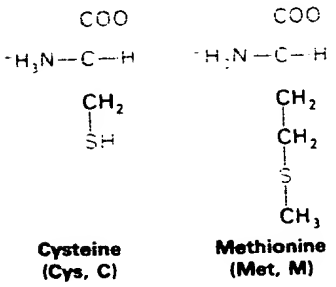


Figure 2-13
Cysteine and methionine have sulfur-containing side chains.

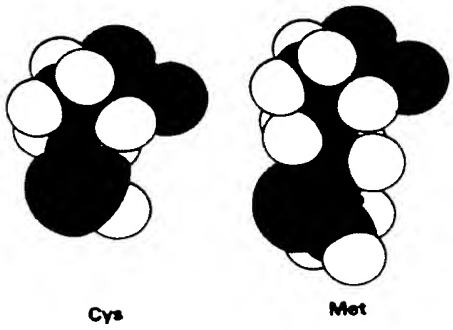


Figure 2-14
Models of cysteine and methionine.

Two amino acids, *serine* and *threonine*, contain aliphatic *hydroxyl groups* (Figure 2-15). Serine can be thought of as a hydroxylated version of alanine, and threonine as a hydroxylated version of valine. The hydroxyl groups on serine and threonine make them much more *hydrophilic* (water-loving) and *reactive* than alanine and valine. Threonine, like isoleucine, contains two centers of asymmetry. All other amino acids in the basic set of twenty, except for glycine, contain a single asymmetric center (the α-carbon atom). Glycine is unique in being optically inactive.

We turn now to amino acids with very polar side chains, which render them highly *hydrophilic*. *Lysine* and *arginine* are *positively charged* at neutral pH. *Histidine* can be uncharged or positively charged, depending on its local environment. Indeed, histidine is often found in the active

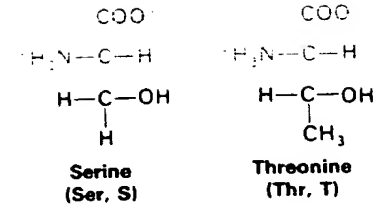


Figure 2-15
Serine and threonine have aliphatic hydroxyl side chains.

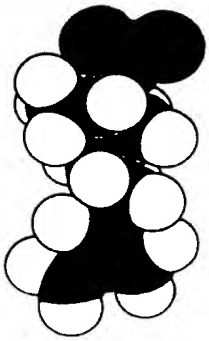


Figure 2-17
Model of arginine. The planar outer part of the side chain, consisting of three nitrogens bonded to a carbon atom, is called a guanidinium group.

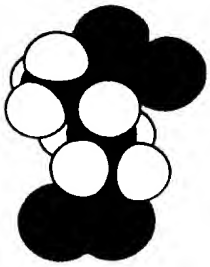


Figure 2-19
Model of glutamate.

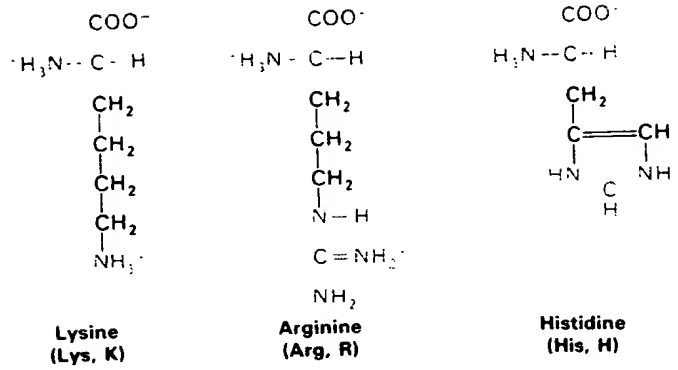


Figure 2-16
Lysine, arginine, and histidine have basic side chains.

sites of enzymes, where its imidazole ring can readily switch between these states to catalyze the making and breaking of bonds. These *basic amino acids* are depicted in Figure 2-16. The side chains of arginine and lysine are the longest ones in the set of twenty.

The repertoire of amino acids also contains two with *acidic side chains*, *aspartic acid* and *glutamic acid*. These amino acids are usually called *aspartate* and *glutamate* to emphasize that their side chains are nearly always negatively charged at physiological pH (Figure 2-18). Uncharged derivatives of glutamate and aspartate are *glutamine* and *asparagine*, which contain a terminal amide group in place of a carboxylate.

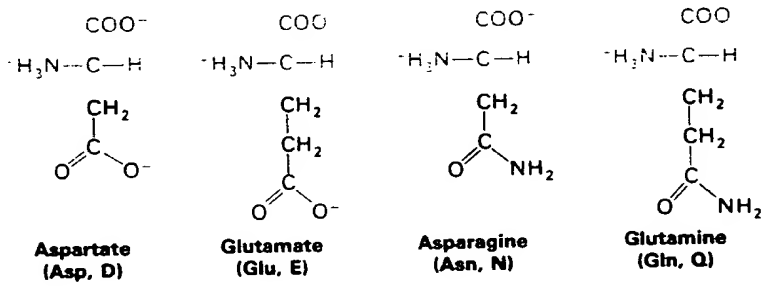
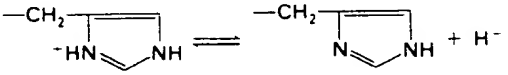
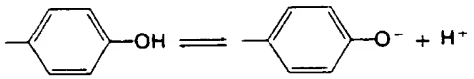
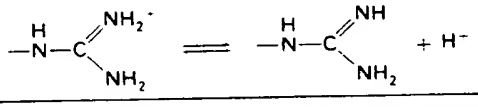


Figure 2-18
Acidic amino acids (aspartate and glutamate) and their amide derivatives (asparagine and glutamine).

Seven of the twenty amino acids have readily ionizable side chains. Equilibria and typical pK_a values for ionization of the side chains of arginine, lysine, histidine, aspartic and glutamic acids, cysteine, and tyrosine in proteins are given in Table 2-1. Two other groups in proteins, the terminal α-amino group and the terminal α-carboxyl group, can be ionized.

Amino acids are often designated by either a three-letter abbreviation or a one-letter symbol to facilitate concise communication (Table 2-2). The abbreviations for amino acids are the first three letters of their names, except for tryptophan (Trp), asparagine (Asn), glutamine (Gln), and isoleucine (Ile). The symbols for the small amino acids are the first letters of their names (e.g., G for glycine and L for leucine); the other symbols have been agreed upon by convention. These abbreviations and symbols are an integral part of the vocabulary of biochemists.

Table 2-1
pK′ values of ionizable groups in proteins

Group	*Acid ⇌ base + H⁺*	*Typical pK**
Terminal carboxyl	$-COOH \rightleftharpoons -COO^- + H^+$	3.1
Aspartic and glutamic acid	$-COOH \rightleftharpoons -COO^- + H^+$	4.4
Histidine		6.5
Terminal amino	$-NH_3^+ \rightleftharpoons -NH_2 + H^+$	8.0
Cysteine	$-SH \rightleftharpoons -S^- + H^+$	8.5
Tyrosine		10.0
Lysine	$-NH_3^+ \rightleftharpoons -NH_2 + H^+$	10.0
Arginine		12.0

*pK′ values depend on temperature, ionic strength, and the microenvironment of the ionizable group.

Table 2-2
Abbreviations for amino acids

Amino acid	*Three-letter abbreviation*	*One-letter symbol*
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V